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Brain Derived Neurotrophic Factor (*BDNF*) Gene Variants and Alzheimer's Disease, Affective Disorders, Posttraumatic Stress Disorder, Schizophrenia, and Substance Dependence

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Genetic variation at the locus encoding the brain derived neurotrophic factor (BDNF) has been implicated in some neuropsychiatric disorders such as Alzheimer's disease (AD), affective disorders (AFDs), schizophrenia, and substance dependence. We therefore performed a mutation scan of the *BDNF* gene to identify novel gene variants and examined the association between *BDNF* variants and several neuropsychiatric phenotypes in European American (EA) subjects and controls. Using denaturing high performance liquid chromatography (dHPLC), we identified a novel variant (G−712A) in the putative promoter region. This variant and two previously reported *BDNF* SNPs (C270T and Val66Met) were genotyped in 295 patients with AD, 108 with AFDs, 96 with posttraumatic stress disorder (PTSD), 84 with schizophrenia, 327 with alcohol and/or drug dependence, and 250 normal control subjects. No association was found between these three *BDNF* gene variants and AD, AFDs, PTSD, or schizophrenia. However, there was a nominally higher frequency of the G−712A G-allele and the G/G genotype in subjects with substance dependence than in controls (Allele: $\chi^2 = 4.080$, df = 1, $P = 0.043$; Genotype: $\chi^2 = 7.225$, df = 2, $P = 0.027$). Although after correction for multiple testing, the findings are not considered significant (threshold *P*-value was set at 0.020 by the program SNPSpD), logistic regression analyses confirmed the modest association between SNP G−712A and substance dependence, when the sex and age of subjects were taken into consideration. The negative results for AFDs, PTSD, and schizophrenia could be due to the low statistical power. Further study with larger samples is warranted.

KEY WORDS: mutation analysis; single nucleotide polymorphism; linkage disequilibrium; association analysis; BDNF

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INTRODUCTION

Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family, promotes neuronal survival and regulates the proliferation and differentiation of nerve cells in the peripheral and central nervous systems [Hartmann et al., 2001]. BDNF has important regulatory effects on the serotonergic [Mossner et al., 2000], glutamatergic [Falkenberg et al., 1996], and dopaminergic [Guillin et al., 2001] neurotransmitter systems. BDNF is also involved in hippocampal long-term potentiation, which is related to learning and memory [Yamada et al., 2002].

There is strong evidence that BDNF may contribute to the pathogenesis of several neuropsychiatric disorders such as Alzheimer's disease (AD), affective disorders (AFDs), and posttraumatic stress disorder (PTSD). For example, reduced BDNF mRNA and protein levels have been found in the hippocampus and other cortical areas in patients with AD [Holsinger et al., 2000]. The production of BDNF in the brains of AFD patients may be deficient as well, since low BDNF levels have been reported in healthy individuals with depressive personality traits [Lang et al., 2004]. Depressed patients who are treated with antidepressants show a significant increase in

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serum BDNF levels [Shimizu et al., 2003]. BDNF is also believed to be involved in PTSD. Rasmusson et al. [2002] observed downregulated BDNF mRNA levels in the hippocampal dentate gyrus of rats exposed to footshock or re-exposed to cues previously paired with footshock. This finding suggests that psychological or physical stress may inhibit hippocampal BDNF expression, which could be relevant to the pathogenesis of stress-related disorders, such as depression and PTSD.

Because of its important role in the development of the dopamine system, which appears to play an important role in the pathophysiology of schizophrenia [Davis et al., 1991], BDNF has also been implicated in that disorder. One study reported elevated BNDF levels in the cingulate cortex and the hippocampus of schizophrenic patients [Takahashi et al., 2000], whereas two other studies showed reduced BDNF in the prefrontal cortex [Weickert et al., 2003] and the hippocampus [Durany et al., 2001] of patients with schizophrenia. Based on the neurodevelopmental hypothesis [Jones and Murray, 1991] and the dopamine theory [Davis et al., 1991] of schizophrenia, BDNF abnormalities may increase vulnerability to the illness. BDNF may also be involved in substance dependence, since the rewarding effects of alcohol and drugs of abuse are largely mediated by serotonergic and dopaminergic systems, and the development of these two neurotransmitter systems is influenced by BDNF. Treatment with and/or withdrawal from drugs [Numan et al., 1998; Grimm et al., 2003] and alcohol [Tapia-Arancibia et al., 2001] may also change brain BDNF expression levels.

Published data support a role for *BDNF* gene variants in some neuropsychiatric disorders. Recently, two *BDNF* polymorphisms (C270T in the non-coding exon I and the missense mutation Val66Met [G196A] in the prepeptide region) have been investigated in AD patients from different populations. Results from three studies suggested a possible effect of C270T [Kunugi et al., 2001; Riemenschneider et al., 2002] and Val66Met [Ventriglia et al., 2002] on the risk of AD. Nevertheless, the three most recent studies have failed to confirm those findings [Bagnoli et al., 2004; Combarros et al., 2004; Tsai et al., 2004]. To identify genetic influences on the risk for bipolar disorder, Sklar et al. [2002] analyzed 96 SNPs in 70 candidate genes in family-based samples and found that only the *BDNF* gene might be a potential risk locus. They observed excessive transmission of the *BDNF* Val(G) allele and a unique haplotype containing the Val(G) allele to affected offspring. Similar results were obtained in another family-based association study [Neves-Pereira et al., 2002]. Furthermore, in a case-control study, Sen et al. [2003] provided evidence of an association between the *BDNF* gene and depression; they found that the less common, non-conserved Met(A) allele was associated with significantly lower mean neuroticism, a personality trait that is frequently elevated in depression [Duggan et al., 1995].

Numerous association studies have examined the *BDNF* gene in schizophrenia, but with conflicting results. Although three studies of a dinucleotide repeat polymorphism (GT)n located about 1 kb upstream from the transcription site of the *BDNF* gene provided suggestive evidence of an association with schizophrenia or negative symptoms of schizophrenia [Krebs et al., 2000; Muglia et al., 2003; Fanous et al., 2004], data from three other studies showed no such association [Sasaki et al., 1997; Hawi et al., 1998; Virgos et al., 2001]. In addition, Nanko et al. [2003] and Szekeres et al. [2003] reported an association between SNP C270T and schizophrenia in the Japanese population.

Only a few studies have examined the association of *BDNF* variants to substance dependence. A genome-wide association analysis, albeit with a low-density marker set, has suggested that markers in proximity to the *BDNF* locus are associated with vulnerability to substance abuse [Uhl et al., 2001]. Three studies reported either no [Matsushita et al., 2004; Tsai et al., 2005] or a suggestive [Liu et al., 2005] association between *BDNF*Val66Met and substance dependence. To our knowledge, there are no published studies of the association of *BDNF* variants with PTSD.

The purpose of the present study was to add to the evidence concerning the association of *BDNF* variants with some psychiatric disorders, and to extend the study of this important locus to other phenotypes and additional variants. We screened polymorphisms in the exons and part of the 5′ region of the *BDNF* gene. A novel gene variant (G−712A) was identified in the putative promoter region. We further analyzed the association of this new marker and two previously reported SNP markers [C270T and Val66Met] with AD, AFDs, PTSD, schizophrenia, or substance (drug and/or alcohol) dependence in European American (EA) subjects.

SUBJECTS AND METHODS

Subjects

All subjects were EAs and all gave informed consent to participate in this study, which was approved by the Institutional Review Board at the relevant institutions. Two hundred ninety-five patients [114 males and 181 females; age: 69.1 ± 8.1 (mean $\pm$ SD)], recruited in the Alzheimer's Disease Research Unit at the Yale University School of Medicine, were diagnosed as having AD according to standard criteria [McKhann et al., 1984]. One hundred eight patients (46 males and 62 females, age information unavailable) with AFDs were recruited at the Connecticut Mental Health Center or the VA Connecticut Healthcare System, West Haven Campus. They were assessed with the structured clinical interview for DSM-III-R (SCID for DSM-III-R) [Spitzer et al., 1992]. Ninety-six subjects [73 males and 23 females; age 43.5 ± 7.2 (mean $\pm$ SD)] diagnosed with PTSD were recruited from the VA Connecticut Healthcare System, West Haven Campus, which is also the location of the Clinical Neuroscience Division of the National Center for PTSD Research. Diagnoses were made using the SCID for DSM-III-R [Spitzer et al., 1992] or the schedule for affective disorders and schizophrenia-lifetime version (SADS-L) [Endicott and Spitzer, 1978], or the PTSD section extracted from the SADS-L. Eighty-four patients [50 males and 34 females; age: 37.5 ± 19.7 (mean $\pm$ SD)] were assessed as having schizophrenia at the VA Connecticut Healthcare System, West Haven Campus. The diagnosis was established using SADS-L or the SCID for DSM-III-R-Patient edition (SCID-P) [Spitzer et al., 1989]. Three hundred twenty-seven subjects [245 males and 82 females; age: 39.8 ± 9.4 (mean $\pm$ SD)] with alcohol and/or drug dependence were recruited at the University of Connecticut Health Center, or the VA Connecticut Healthcare System, West Haven Campus. Diagnoses were made using the SCID for DSM-III-R [Spitzer et al., 1992], the computerized diagnostic interview schedule for DSM-III-R (C-DIS-R) [Blouin et al., 1988], or a checklist comprised of DSM-III-R symptoms. Among the 327 substance dependent patients, 265 had alcohol dependence (44.9% had comorbid drug dependence) and 179 had drug (cocaine and/or opiate) dependence (67.6% had comorbid alcohol dependence). Additionally, 250 control subjects [103 males and 147 females; age: 37.5 ± 19.7 (mean $\pm$ SD)] came from the normal healthy EA population. They were screened, either by structured interview or through an unstructured evaluation, to exclude the above-mentioned conditions.

Mutation Analysis

Brain derived neurotrophic factor sequence information was obtained through the public "GoldenPath" database (http://genome.ucsc.edu). Fifteen pairs of primers were designed (based on the nucleotide sequence of hg13_refGene_

NM_001709 range = chr11:28453035–28497937) to amplify part of the 5′ region, the non-coding exon I, and the only coding exon V of the *BDNF* gene (see online Supplement I at http://www3.interscience.wiley.com/cgi-bin/jhome/99018626/). Amplicons derived from 16 to 48 DNA samples of the above patients with various diagnoses and healthy controls were scanned using denaturing high performance liquid chromatography (dHPLC). A dHPLC mutation screen of the *BDNF* gene was carried out by using the "Wave" DNA Fragment Analysis System (Transgenomic, Inc., Omaha, NE). Five-15 μl of the polymerase chain reaction (PCR) mixture was loaded on a "DNASep" cartridge using a linear acetonitrile gradient in a triethylamine buffer at a constant flow rate of 0.9 ml/min. The gradient was created by mixing the eluents A (0.1 M triethylamine) and B (0.1 M triethylamine and 25% Acetonitrile) at concentrations determined by the Transgenomic "Wavemaker" software. Samples that showed mobility shifts were sequenced using cycling sequencing and fluorescently labeled dideoxynucleotides on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Markers and Genotyping

A newly identified SNP, G−712A, and two previously reported SNPs, C270T [Kunugi et al., 2001] and Val66Met [Nanko et al., 2003], were genotyped in patients with AD, AFDs, PTSD, schizophrenia, substance (alcohol and/or drug) dependence, and in normal control subjects by PCR and restriction fragment length polymorphism (PCR-RFLP) analysis. Primer pairs and PCR conditions for genotyping the three *BDNF* SNPs can be found in the online Supplement II (http://www3.interscience.wiley.com/cgi-bin/jhome/99018626/).

Statistical Analysis

The heterozygosity (H) rate for each marker was calculated by the formula $H = 1-\Sigma P_i^2$, with P_i denoting the frequency of the ith allele. The presence of Hardy–Weinberg equilibrium (HWE) for genotypic distributions was examined using the χ^2-test for goodness of fit. Linkage disequilibrium (LD) values between alleles of G−712A, C270T, and Val66Met were obtained using the expectation-maximum (EM) method, which is implemented in the program 3LOCUS [Long et al., 1995]. The obtained test statistic G reflects the likelihood ratio of the specified model (LD between SNPs) to the restricted model (null hypothesis of no disequilibrium). To analyze the significance level of LD, a series of G values was assessed using Monte-Carlo simulations. The *P*-value was then estimated as the proportion of times that the simulated G reached or exceeded the G calculated from the actual data. The strength of LD between SNPs was also presented as values of D' (the normalized LD statistic).

The differences in genotype and allele frequencies for these three markers between cases and controls were evaluated by the χ^2-test. To correct for multiple statistical tests, the program SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), which takes marker LD information into consideration [Nyholt, 2004], was used to generate the experiment-wide significance threshold required to keep the Type I error rate at <5%. To examine whether sex and age differences between cases and controls could confound the association results, a backward stepwise logistic regression analysis was performed, with diagnosis as the dependent variable, and sex, age, and allele or genotype information as independent variables. Both χ^2 tests and logistic regression analyses were carried out using SPSS 13.0 for Windows (http://www.spss.com). Haplotype frequencies were estimated using the program PHASE, version 2.1 [Stephens and Donnelly, 2003], which was also used to perform a permutation test for differences in haplotype frequencies between cases and controls. Sample sizes and the power of case-control studies were calculated using the online program: Power Calculator (http://calculators.stat.ucla.edu/powercalc/binomial/case-control/).

RESULTS

Mutation Detection at the *BDNF* Locus

Using dHPLC and direct sequencing, we identified a novel SNP (G/A) in the putative promoter region of the *BDNF* gene. It is located 712 bp upstream of the first exon of the *BDNF* gene (here designated as "G−712A"). In addition, one known SNP in exon 1 (C270T) and two known SNPs causing isoleucine (2) to threonine and valine (66) to methionine amino acid changes, respectively, in the coding sequence were detected. The Ile2Thr missense variant was found to be rare (frequency lower than 0.01 in the general population).

Association Analysis of Individual *BDNF* SNPs

The newly identified SNP, G−712A, plus C270T and Val66Met, were genotyped in 250 unrelated EA controls and five groups of EA cases affected with AD (n = 295), AFDs (n = 108), PTSD (n = 96), schizophrenia (n = 84), or substance dependence (n = 327). The heterozygosity rates for G−712A, C270T, and Val66Met in the EA control population were estimated to be 0.12, 0.12, and 0.31, respectively. The genotypic distribution of these three SNPs for all five groups of cases and the controls were consistent with HWE expectations.

As shown in Table I, no significant differences in allele or genotype frequencies of the three *BDNF* SNPs were found between the normal controls and the four groups of patients affected with AD, AFDs, PTSD, or schizophrenia. The only positive finding was that the −712G allele and the −712G/−712G homozygote were significantly more frequent in substance dependent patients than in controls (Allele: $\chi^2 = 4.080$, df = 1, $P = 0.043$; Genotype: $\chi^2 = 7.225$, df = 2, $P = 0.027$). When the two subgroups of substance dependent subjects (265 alcohol and 179 drug dependent subjects) were considered separately, SNP G−712A showed a nominally significant association with alcohol dependence (Allele: $P = 0.045$; Genotype: $P = 0.024$) and a suggestive association with drug dependence (Allele: $P = 0.096$; Genotype: $P = 0.087$). After adjusting for multiple testing (threshold significance *P*-value was set at 0.020 by the program SNPSpD), these results were not considered statistically significant. However, backward stepwise logistic regression analyses confirmed that the −712G allele might be a susceptibility factor for substance dependence (−712G allele: $\beta = 2.062$, $P = 0.009$, odds ratio = 2.062; −712G/−712G genotype: $\beta = 1.031$, $P = 0.009$, odds ratio = 2.804), when sex and age information of subjects were taken into consideration. Additionally, inclusion of sex and age as factors in logistic regression analyses did not modify the absence of an association for the three SNP variants in AD, AFDs, PTSD, or schizophrenia.

Pairwise LD Between *BDNF* SNPs

As summarized in Table II, close LD ($P < 0.001$) was found between SNPs G−712A (in the putative promoter region) and C270T (in non-coding exon I) in all five case groups and the control group. SNP Val66Met (in coding exon V) and SNP G−712A or SNP C270T were in modest LD in the control group (Val66Met/G−712A, $P = 0.016$; Val66Met/C270T, $P = 0.011$) and in patients affected with AD (Val66Met/G−712A, $P = 0.019$; Val66Met/C270T, $P = 0.003$) or schizophrenia (Val66Met/G−712A, $P = 0.019$; Val66Met/C270T, $P = 0.023$). However, SNP Val66Met was not found to be in LD with either SNP G−712A or SNP C270T in the other three patient groups (AFDs, PTSD, or substance dependence).

TABLE I. Comparison of Allele and Genotype Frequencies of *BDNF* SNPs in Cases and Controls

BDNF SNPs	Controls (n = 250)	Alzheimer's disease (n = 295)	Affective disorders (n = 108)	Posttraumatic stress disorder (n = 96)	Schizophrenia (n = 84)	Substance dependence (n = 327)
G−712A						
G	0.938	0.954	0.917	0.948	0.917	0.966
A	0.062	0.046	0.083	0.052	0.083	0.034
P-value (allele)		0.292	0.381	0.753	0.437	0.043[a]
G/G	0.876	0.915	0.852	0.896	0.833	0.935
G/A	0.124	0.078	0.130	0.104	0.167	0.062
A/A	0.000	0.007	0.018	0.000	0.000	0.003
P-value (genotype)		0.090	0.095	0.745	0.420	0.027[b]
C270T						
C	0.940	0.956	0.926	0.932	0.940	0.948
T	0.060	0.044	0.074	0.068	0.060	0.052
P-value (allele)		0.294	0.590	0.841	0.869	0.646
C/C	0.880	0.919	0.870	0.865	0.881	0.896
C/T	0.120	0.074	0.111	0.135	0.119	0.104
T/T	0.000	0.007	0.019	0.000	0.000	0.000
P-value (genotype)		0.089	0.096	0.836	0.864	0.636
Val66Met						
A(Met)	0.188	0.214	0.167	0.146	0.196	0.174
G(Val)	0.812	0.786	0.833	0.854	0.804	0.826
P-value (Allele)		0.331	0.566	0.233	0.899	0.600
A/A	0.040	0.031	0.028	0.010	0.036	0.022
A/G	0.296	0.366	0.278	0.271	0.321	0.304
G/G	0.664	0.603	0.694	0.719	0.643	0.674
P-value (genotype)		0.209	0.779	0.306	0.902	0.477

n, number of individuals.
P-value: Chi-square test *P*-value.
[a]$\chi^2 = 4.080$, df = 1, $P = 0.043$.
[b]$\chi^2 = 7.225$, df= 2, $P = 0.027$.

Haplotype Association Analysis

Six haplotypes consisting of alleles of the three *BDNF* SNPs were observed. One major haplotype [−712G−270C−Val66(G)] with frequency of about 75% was found in all six groups of samples. The second most frequent haplotype was −712G−270C−Val66(A), which accounted for 15–20% of all haplotypes. Other haplotypes were rare. As can be seen in Table III, using the case-control study option in the PHASE program to compare haplotype frequencies between the five case groups and the control group revealed no significant differences.

DISCUSSION

In the present study, we screened part of the 5′ putative regulatory region, the non-coding exon I and the only coding exon V of the *BDNF* gene for novel variants in a sample comprised of EA normal controls and patients affected with AD, AFDs, PTSD, schizophrenia, or alcohol and/or drug dependence. A new SNP variant (G−712A) was identified in the 5′ region of the *BDNF* gene (712 bp from exon I). Both the Genomatix (http://www.genomatix.de) and the Neural Network Promoter Prediction software (http://darwin.nmsu.edu/~molb470/fall2003/Projects/solorz/neural_network_promoter_predicti.htm) predict that the sequence of this region is potentially a part of the eukaryotic polymerase II promoter binding site, and that the variant allele of the newly discovered polymorphism may disrupt the pattern of recognition. This implies a possible negative effect of this variant on transcription of the *BDNF* gene. Accordingly, the new SNP, G−712A, and two other reported SNPs, C270T (in exon I) and Val66Met (a missense mutation in the coding exon V), were genotyped in the patient groups and in normal control subjects. By logistic regression analysis, we observed an association between the

TABLE II. Pairwise Linkage Disequilibrium (LD) Between *BDNF* SNPs in Cases and Controls

		Controls	Alzheimer's disease	Affective disorders	Posttraumatic stress disorder	Schizophrenia	Substance dependence
SNP pairs							
G−712A/C270T	D'	0.941	1.000	0.939	0.972	0.977	0.877
	G	120.14	175.20	86.89	52.28	92.11	86.18
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G−712A/Val66Met	D'	0.642	1.000	1.000	0.146	0.445	1.000
	G	5.98	6.07	3.12	0.02	5.11	1.45
	P	0.016	0.019	0.084	0.877	0.019	0.255
C270T/Val66Met	D'	0.627	1.000	1.000	0.173	0.445	1.000
	G	6.21	8.00	2.21	0.05	4.84	3.94
	P	0.011	0.003	0.157	0.519	0.023	0.059

D', normalized linkage disequilibrium statistic.
G, G-value.
P, significance level.

TABLE III. Comparison of *BDNF* Haplotype Frequencies in Cases and Controls

BDNF haplotypes	Controls (n = 250)	Alzheimer's disease (n = 295)	Affective disorders (n = 108)	Posttraumatic stress disorder (n = 96)	Schizophrenia (n = 84)	Substance dependence (n = 327)
G−712A−C270T−Val66Met						
G-C-A	0.186	0.213	0.167	0.146	0.196	0.172
G-C-G	0.750	0.741	0.746	0.797	0.721	0.774
G-T-G	0.002	0.000	0.005	0.005	0.000	0.016
A-C-G	0.004	0.002	0.005	0.005	0.000	0.004
A-T-A	0.002	0.000	0.000	0.000	0.000	0.000
A-T-G	0.056	0.044	0.077	0.047	0.083	0.033
P-value for testing H_0:		0.544	0.900	0.270	0.950	0.655

n, number of individuals.
P-value for testing H_0 (no association) was computed by PHASE program (20,000 permutations).

newly described SNP G−712A and substance dependence (A nominally-significant marker association analysis result did not withstand correction for multiple comparisons). No evidence of association was found between any of the three gene variants and the other four disorders.

All five disorders studied are complex traits, presumably influenced by multiple genes with minor or moderate effects. Power analysis demonstrated that our study had a statistical power of 94.2% (for AD), 75.1% (for ADF), 71.5% (for PTSD), 67.3% (for schizophrenia), and 95.1% (for substance dependence) to detect a significant association ($P < 0.05$, two-sided), assuming frequency of the risk allele to be 0.20 and a relative risk ratio of 2. In other words, if the less frequent alleles of the three SNP markers were risk alleles, our study was adequately powered to detect a significant association only between SNP Val66Met (the frequency of the Met66(A) allele varied from 14.6% to 21.4%) and AD or substance dependence. As we did not find an association between SNP Val66Met and AD, it is unlikely that this polymorphism plays a major role in the pathogenesis of this condition in the EA population. The conclusion regarding the Val66Met variant in AD is consistent with previous findings in Chinese [Tsai et al., 2004], Spanish [Combarros et al., 2004], and Italian [Bagnoli et al., 2004] populations. Given the low heterozygosity or the low information content of SNPs C270T and G−712A (the frequency of their less common allele was only about 5%), these two polymorphisms appear to require larger numbers of cases to ensure adequate statistical power. In addition, although our overall study sample was large (total N for this study = 1,160), the samples for some of the phenotypes (AFDs, PTSD, and schizophrenia) were modest, and this limited our power to detect significant associations. This may also partially explain the non-significant results in haplotype analyses. Therefore, larger "affected" samples are needed to verify the negative findings in this study.

False positive or negative results in case-control studies may also result from population stratification. Under such circumstances, the risk allele may have different frequencies in different ethnic groups or the specific allele may be a risk factor for one population but not others. In the present study, to reduce the risk of ethnic biases, all case and control subjects were of European ancestry. Tang et al. [2005] have shown that genetic clustering for several groups, including EAs, corresponds very closely to racial self-identification; therefore, it is unlikely that our negative findings are due to population stratification. Although sex and age may confound association analyses, in this study, they were found not to influence the negative association results of the three gene variants in AD, AFDs, PTSD, or schizophrenia.

Another possible explanation for the negative results in AD, AFDs, PTSD, and schizophrenia is that either the *BDNF* gene does not contribute to risk of these disorders or the three studied SNPs are not in LD with a functional polymorphism (as yet unidentified) that lies near or in the *BDNF* gene. In the present study, we found close LD among the three *BDNF* SNP markers in normal controls and in patients with AD or schizophrenia, even though SNP Val66Met is about 40 kb away from SNPs G−712A and C270T. It is also worth noting that there was limited haplotypic diversity over the *BDNF* gene, as defined by these markers (the major haplotype, −712G−270C−Val66(G), had a frequency of around 75%). The haplotype data also indicated the presence of high-level LD between markers in the *BDNF* gene. Two other studies reported similar results. Sklar et al. [2002] tested eight SNPs covering the whole *BDNF* gene region in patients with bipolar disorder and Hall et al. [2003] tested 10 SNPs covering the whole *BDNF* gene region in patients with obsessive-compulsive disorder (OCD). They found extensive background LD at this locus. Strong LD was observed between SNPs within the *BDNF* gene [Sklar et al., 2002; Hall et al., 2003] and those located in both upstream and downstream regions [Sklar et al., 2002], or only in the upstream region [Hall et al., 2003] of the *BDNF* gene. That both the present and the two prior studies indicated strong LD for markers in the *BDNF* gene region implies that, if these three SNPs were susceptibility loci themselves or in close LD with a real disease locus in the *BDNF* gene, they should have shown an association with the disorders we studied.

The lack of association of SNP C270T with substance dependence may reflect the relatively lower LD (D' = 0.877) between this marker and SNP G−712A in substance dependent subjects than in other groups of subjects. Although we did not find strong LD between SNP Val66Met and SNP G−712A or SNP C270T in cases affected with AFDs, PTSD, or substance dependence, this is of uncertain significance, and may be due to the limited information content for some of the markers or the small size of the samples (for AFDs and PTSD).

Alternatively, disorders like AFDs represent a genetically heterogeneous group, which may make it difficult to identify associations. Therefore, it would be worthwhile to examine whether *BDNF* gene variants affect a specific trait (e.g., memory and hippocampal dysfunction) in patients with these disorders rather than the disorders per se. In addition, as the gene variant G−712A was found to be associated with substance dependence and is located in the putative promoter region, it may be of interest to investigate its effects on transcription of the *BDNF* gene.

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